



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁴ : G01N 27/00	A1	(11) International Publication Number: WO 88/08972 (43) International Publication Date: 17 November 1988 (17.11.88)
(21) International Application Number: PCT/US88/01494 (22) International Filing Date: 4 May 1988 (04.05.88)		(74) Agent: CROUSE, Daniel, D.; Christensen, O'Connor, Johnson & Kindness, 2700 Westin Building, 2001 Sixth Avenue, Seattle, WA 98121 (US).
(31) Priority Application Number: 047,080 (32) Priority Date: 5 May 1987 (05.05.87) (33) Priority Country: US		(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).
(71) Applicant: THE WASHINGTON TECHNOLOGY CENTER [US/US]; University of Washington, Loew Hall, M.S. FH-10, Seattle, WA 98195 (US). (72) Inventors: CHEUNG, Peter, Wing-Poon ; 7865 West Mercer Way, Mercer Island, WA 98040 (US). WEILER, Edward, Bakewell ; 2850 N.W. 64th, Seattle, WA 98107 (US). FURLONG, Clement, Eugene, Jr. ; 18256 - 57th N.E., Seattle, WA 98155 (US).		Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: A SYSTEM EMPLOYING A BIOSENSOR TO MONITOR A CHARACTERISTIC OF A SELECT COMPONENT IN A MEDIUM		<p>A measuring instrument is disclosed having a reversibly selective binding protein immobilized upon the insulated-gate region of a field-effect transistor located on a sensor. With the sensor immersed in solution, the protein binds a select component of the solution to the gate producing an effect on a current flowing through the IGFET. A plurality of such binding protein-IGFET arrangements can be provided on the same sensor, including the same binding proteins having different binding coefficients K_D or an array of proteins with different ligand specificity and/or affinity. Analysis of the IGFET's response to binding by a microprocessor allows, for example, the concentration of the component in solution to be determined. With a plurality of different binding proteins employed, the concentration of different components can be determined. Similarly, with binding proteins employed having different binding coefficients K_D, the output of the sensor can be analyzed in either a digital or analog manner, or some combination of the two. To release the component from the protein, heat can be applied to the sensor through a resistive bank integrated into the sensor chip. Alternatively, the application of a reverse bias to the gate can be used to enhance release.</p>

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT Austria	FR France	ML Mali
AU Australia	GA Gabon	MR Mauritania
BB Barbados	GB United Kingdom	MW Malawi
BE Belgium	HU Hungary	NL Netherlands
BG Bulgaria	IT Italy	NO Norway
BJ Benin	JP Japan	RO Romania
BR Brazil	KP Democratic People's Republic of Korea	SD Sudan
CF Central African Republic	KR Republic of Korea	SE Sweden
CG Congo	LI Liechtenstein	SN Senegal
CH Switzerland	LK Sri Lanka	SU Soviet Union
CM Cameroon	LU Luxembourg	TD Chad
DE Germany, Federal Republic of	MC Monaco	TG Togo
DK Denmark	MG Madagascar	US United States of America
FI Finland		

-1-

A SYSTEM EMPLOYING A BIOSENSOR TO MONITOR
A CHARACTERISTIC OF A SELECT COMPONENT IN A MEDIUM

Field of the Invention

This invention relates to measuring systems employing sensors
5 and, more particularly, to measuring systems employing sensors to monitor a
select component of a medium.

Background of the Invention

It is often desirable to monitor particular characteristics of a
certain component of interest found in a medium. For example, the monitoring
10 of phosphate levels in sewage is useful to determine whether the sewage has
been adequately treated. Similarly, in virtually any chemical processing system,
knowledge of the concentration of a particular component in solution can be
useful for system control. Further, in biomedical applications, information
concerning the concentration of certain components in body fluids such as blood
15 can be extremely important to the proper diagnosis of a patient's condition.

Given the diversity of characteristics, components, and media
that may be involved, a system, including a measuring instrument and a sensor,
that is able to accurately produce measurements under these different conditions
is subject to numerous design constraints. For example, the system may be
20 required to provide a selective response to a particular characteristic and
component of the media. The system may also be called upon to automatically
respond to changes in the characteristic of the component, providing updated
information with minimal operator intervention. In certain applications, the
system sensor preferably would have a relatively long life and be reusable

-2-

without adverse effect upon its accuracy. Alternatively, it may be desirable in some instances to employ a sensor that is disposable. Finally, it would be desirable to produce a system that is either precalibrated, or requires no calibration, and whose size, complexity and cost are kept to a minimum.

5

Summary of the Invention

In accordance with this invention, a system is provided for detecting a characteristic of a component of a medium. The system includes a sensor having a detector that responds electronically when exposed to the component and a binding element for binding the component to the detector.

10 The instrument also includes a processor that is coupled to the sensor and produces an output indicative of the characteristic of the component of the medium.

In a preferred arrangement, the binding element reversibly binds the component to the detector, the medium is a solution and the characteristic of interest is the component's concentration in the solution. The binding element is an organic or inorganic ligand binder.

In accordance with a particular aspect of the invention, the detector is a semiconducting material comprising an insulated gate field-effect transistor. The transistor has a gate insulator region, source region and drain region. The organic or inorganic ligand binder is coupled to the gate insulator region, while the processor applies an electronic signal between the source region and drain region of the transistor. The binding of the component to the gate insulator region influences the signal and the processor responds by producing an output indicative of the characteristic of the component.

25

In accordance with a further aspect of the invention, a plurality of insulated gate field-effect transistors are employed, with a separate organic or inorganic ligand binder being coupled to the gate region of each transistor. The separate organic or inorganic ligand binders may bind the same components and have the same or different binding coefficients, or may bind different components.

30

In accordance with another aspect of this invention, a method of detecting a characteristic of interest of a component of a medium is provided. The method includes the step of binding the component to a semiconducting element that responds electronically to the component. Then, the electronic

response of the semiconducting element is monitored and an output indicative of the characteristic of interest is produced, the output being a function of the electronic response monitored.

Brief Description of the Drawings

5 The invention will presently be described in greater detail, by way of example, with reference to the accompanying drawings, wherein:

10 FIGURE 1 is a pictorial view of a measuring system constructed in accordance with this invention, including a sensor, detachably connected to a console that controls the sensor and responds to information received from the sensor;

FIGURE 2 is a pictorial view of an alternative embodiment of the measuring system shown in FIGURE 1, including a discrete sensor;

FIGURE 3 is a block diagram of the measuring system illustrated in FIGURES 1 and 2;

15 FIGURE 4 is a more detailed block diagram of the measuring system illustrated in FIGURE 3;

FIGURE 5 is a pictorial representation of a portion of a sensor constructed in accordance with this invention;

20 FIGURE 6 is a schematic representation of the sensor represented in FIGURE 4;

FIGURE 7 is a pictorial representation of a sensor, constructed in accordance with this invention, having a plurality of sensing regions; and,

25 FIGURE 8 is a graphical illustration of the response of a particular sensor, constructed in accordance with this invention, to the changing concentration of a component in solution.

Detailed Description of the Preferred

Embodiment of the Invention

Referring now to FIGURE 1, a measuring system 10 constructed in accordance with this invention is illustrated. As shown, measuring system 10 includes an instrument console 12 and a detachable sensor assembly 14. The distal portion of sensor assembly 14 includes a sensor element 16, which is exposed to the media containing the component of interest. The sensor element 16 is electronically and mechanically coupled to console 12 by a cable 18. A connector 20, provided at the proximal end of cable 18, allows sensor assembly 14 to be detachably connected to console 12.

-4-

The operation of sensor assembly 14 is controlled, in part, by instructions introduced through a user input such as a keypad 22 provided on the instrument console 12. The internal circuitry of console 12 monitors the particular characteristic of the component of interest by analyzing signals from sensor element 16 in the manner described in greater detail below. The resultant information is then displayed to an operator via display 24 and can also be used in a feedback control system to adjust system parameters.

In an alternative to the embodiment of the measuring system 10 shown in FIGURE 1, the instrument console 12 is dimensioned to be handheld and is used with a discrete sensor element 16 cassette. As shown in FIGURE 2, the sensor element 16 is insertable into console 12, which electrically and mechanically engages the sensor element 16 in a manner described in greater detail below.

Like the embodiment of system 10 shown in FIGURE 1, the operation of the discrete sensor element 16 is controlled, in part, by instructions introduced through a user input, such as a keypad 22, provided on the instrument console 12. A particular characteristic of the component of interest is monitored via console 12 by the analysis of signals from sensor element 16, as described below. The information produced by console 12 is then displayed to the operator via a display 24.

A basic block diagram of the measuring systems 10 of FIGURES 1 and 2 is shown in FIGURE 3. As shown, sensor assembly 14 is controlled by, and provides information to, the processing circuit 26 located within console 12. The operation of processing circuit 26 is controlled, in part, by operator instructions received via input elements 28. Display elements 30 convert information from processing circuit 26 into a form usable by the operator. Similarly, control elements 32 convert information about the characteristic of interest received from processing circuit 26 into forms usable by a feedback control system.

Turning now to a more detailed description of the various components of measuring system 10, the detachable sensor assembly 14 is first considered. As shown in the more detailed block diagram of system 10 provided in FIGURE 4, sensor element 16 includes a binding element 32 immobilized on a semiconducting element 34. The function of binding element 32 is preferably to selectively and reversibly bind the component of interest to the semiconducting

element 34 when sensor element 16 is exposed to, for example, a solution. As will be discussed in greater detail below, the binding of certain select components to the semiconducting element 34 induces a measurable response in semiconducting element 34. As a result, the concentration or activity of the component in solution, for example, can be monitored as a function of the electronic characteristics of the semiconducting element 34. The electronic response of semiconducting element 34 is applied to a conditioning circuit 36 which may also be included on sensor element 16. As described below, circuit 36 appropriately conditions the response of semiconductor element 34 for transmission to processing circuit 26 via cable 18. A binding reversal element 38 is additionally included on sensor element 16 to reverse the action of the binding element 32.

Addressing these components of sensor element 16 in greater detail, binding element 32 is preferably a biologically active molecule immobilized on the semiconducting element 34. Such biologically active molecules are selected from proteins, protein fragments and binding partners that are capable of forming reversible binding complexes with the component, which is typically a small molecule ligand. The preferred biologically active molecules are a class of proteins termed "binding proteins" that reside in the space between the inner and outer membranes (periplasmic space) of the gram-negative prokaryotes. These proteins bind their respective nutrients with high specificity and affinity. These proteins also bind their respective ligand through a broad range of pH values and ionic strengths.

It is known that protein ligand binding causes a conformational change in the protein (Ames, G.F.L., Ann. Rev. Biochemistry, 1986, 55:397-425) resulting in a tight binding complex. Binding affinities (K_D) for ligand binding proteins are described in Furlong, C.E., Methods in Enzymology 125: 279-289 (1986), hereby incorporated by reference. The high ligand affinity of these proteins is directly related to a low dissociation rate, requiring that these proteins be "encouraged" to release their ligand by external stimulus to prevent the sensor from remaining saturated. It has been discovered in accordance with the present invention that prokaryote periplasmic binding proteins which have formed a high affinity complex with a ligand will release the ligand upon heating (for example to temperatures above 75°C for the phosphate-binding protein) and

-6-

that the binding protein will renature to its original conformation upon cooling to ambient temperature, provided that the binding protein is immobilized on a surface prior to heating. If the protein is not immobilized, denaturing by heating causes irreversible protein coagulation. Thus, these binding proteins may be 5 suitably employed in a sensor for detecting low concentrations (near the K_D) of ligands in aqueous solution and they may be cycled by heating and cooling or other reversibly denaturing conditions such as organic solvents and chaotropic agents to release the bound ligand and regenerate the active sensor.

As will be appreciated, the ligand of interest may be a charged or 10 uncharged species. The corresponding binding protein may be selected from those described by Furlong, as referenced above. Still other proteins suitable for use include those described by Copeland, B.R. et al., J. Biol.Chem. 257 15065-15071 (1982), herein incorporated by reference.

In general, the biologically active molecule need only bind its 15 ligand with an affinity and specificity appropriate to the particular application. A general procedure for identifying and isolating proteins is described by Copeland, B.R. et al., referenced above. The entire protein may not be necessary if an active binding domain of a protein can be isolated and if the domain can be reversibly denatured.

It is preferred that the K_D of the binding protein be near the 20 concentration of the solution being analyzed. If the concentration of ligand in solution is higher than the K_D of the protein, the measuring system can employ a feedback controlled dilution system to adjust, calculate and control the necessary dilution of the original solution to bring it within measurement range 25 for a given binding protein attached to the semiconducting element 34.

The means for immobilizing a binding component 32 on semiconducting element 34 depends on the composition of semiconducting element 34. When the binding protein is immobilized on a silicon dioxide region at the surface of semiconducting element 34, described in greater detail below, 30 the silicon dioxide surface is preferably derivatized using vapor phase deposition of silane compounds, such as 3-aminopropyltriethoxysilane (APTES). Vapor phase deposition in this manner deposits a chemically active layer of the silane compound covalently bonded to the SiO_2 surface. Alternatively, the surface can be derivatized using liquid phase deposition.

-7-

A binding protein, selected from those previously described, is then immobilized on the derivatized surface of the semiconductor by a covalent bond. Bonding the protein to the derivatized SiO₂ surface must be conducted under conditions that preserve the biological activity of the binding protein.

5 Bifunctional cross-linkers, of the type disclosed in the 1986-87 catalog of the Peirce Chemical Company, Rockford, Illinois, pages 312-340, herein incorporated by reference, are suitable.

Turning now to a more detailed description of semiconducting element 34, reference is had to FIGURE 5. As shown, semiconducting element 34 is preferably configured as an insulated-gate, field-effect transistor (IGFET) 40. As noted previously, the function of semiconducting element 34 is to respond electronically to the action of binding element 32. The IGFET configuration is preferred for its extremely high input impedance, which limits the influence that semiconducting element 34 has on binding element 32.

15 As shown in the representation of IGFET 40 provided in FIGURE 5, IGFET 40 is constructed for operation in an n-channel depletion-mode. More particularly, IGFET 40 includes a substrate 42 formed of a semiconducting material, such as silicon, that has a p-type impurity added to increase the number of positive charge carriers, or holes, present in the material. Separate source 44 and drain 46 regions are provided at spaced-apart locations on the surface of substrate 42. While the source 44 and drain 46 are also preferably made of a silicon semiconducting material, they are heavily doped with an n-type impurity that significantly increases the number of negative charge carriers, or electrons, present in the material. A channel 48 of semiconducting material extends between source 44 and drain 46 and is lightly doped with an n-type impurity to provide a relatively low conductivity. An insulative material 50, such as silicon dioxide, is deposited over channel 48 to produce the high input impedance noted above. A gate region 52 of IGFET 40 is defined by the insulating material 50 and, in this case, has the previously described binding element 32 immobilized thereon. A gate electrode 53 is spaced apart from the gate region 52 and allows a reference voltage to be applied to the gate region 52. Ohmic contacts 54 allow the substrate 42, source 44 and drain 46 regions of IGFET 40 to be connected to external circuitry, while the remainder is insulated by dielectric material 55.

-8-

Operation of IGFET 40 is basically as follows. With the source 44 and substrate 42 electronically connected and no voltage applied to the gate insulator 50 via reference electrode 53, the application of a small positive voltage V_{DS} between the drain 46 and source 44 establishes a reverse-bias across 5 the p-n junction. The reverse bias forms a depletion region between the channel 48 and substrate 42 that effectively isolates the two regions. With IGFET 40 operated in this manner, current flows from source 44 to drain 46 through the channel 48, which acts as a resistor whose resistance depends on the physical construction of channel 48.

Now, assume that binding element 32 has been constructed to 10 selectively bind a negatively charged component in solution to gate insulator 50. The resultant negative potential, which may be supplemented by an external gate-to-source voltage via electrode 53, produces a field at the insulative material 50 that repels electrons in the n-type material of channel 48. As a 15 result, the conductivity of channel 48 decreases and, at some point, V_p , the magnitude of the gate-to-source voltage V_{GS} may become sufficiently large to effectively "pinch off" channel 48, preventing the flow of drain current I_D . Below pinch-off and for relatively low drain-to-source voltages V_{DS} (less than $V_{GS} - V_p$), IGFET 40 effectively acts as a voltage-controlled linear resistance, 20 with the gate potential produced by the operation of binding element 32 and any external source controlling resistance. For larger values of V_{DS} , however, the drain current I_D varies only with V_{GS} and is not a function of V_{DS} .

As will be appreciated, an IGFET 40 constructed in the foregoing manner can be used to detect the concentration of the component in solution in 25 the following manner. With the appropriate binding element 32 immobilized upon insulative material 50 and some reference to the substrate 42 provided, V_{GS} is a function of the number of negatively charged components bound to insulative material 50. With a constant voltage V_{DS} applied from processing circuit 26, the drain current I_D is inversely proportional to the magnitude of V_{GS} 30 and, hence, the concentration of the negatively charged component in solution.

Although semiconducting element 34 has been described as an n-channel, depletion-mode IGFET constructed of silicon with a silicon dioxide insulative layer, various other configurations could be employed. For example, IGFET 40 could be constructed for use with a p-type channel as well as for use in

an enhancement-mode. Alternative semiconducting materials that can be used in IGFET 40 include Ge, SiC, silicon-on-sapphire and GaAs, while other usable insulating materials include Si_3N_4 , Al_2O_3 , tantalum oxide, and titanium oxide. Alternatively, those skilled in the art will appreciate that certain metals such as 5 aluminum, antimony, chrome, gold, platinum, silver and metal/metal oxide systems of these metals can be used to construct metal and/or metal/metal oxide electrodes which could be employed to measure conductance, resistance or potentiometric changes in response to the operation of the binding element 32 in solution. Further, it is contemplated that constructions employing junction 10 field-effect transistors, bipolar transistors, gate-controlled diodes, capacitors, charge coupled devices, diodes, and unijunction transistors could be employed, with the charge generation, charge redistribution, change in dipole moment, change in conductivity, or change in capacitance caused by the operation of binding element 32 upon the component in solution producing an electronic 15 response that can be correlated to, for example, the concentration of the component.

In the particular IGFET 40 construction represented in FIGURE 4, IGFET 40 preferably has a gate region 52 whose dimensions are on the order of 10 microns by 200 microns and a substrate resistivity of six to ten ohms-centimeter. The depth of the silicon dioxide insulative layer 50 is 20 approximately 500 to 1000 Å and has a surface area corresponding to that of gate region 52.

With the binding proteins 32 immobilized on the insulative material 50 in the manner described above, a protein density per square centimeter of gate insulator region 52 of approximately 10^{13} can be achieved. 25 In this manner, when a protein 32 having a K_D of one is employed, and the proteins 32 have completed their binding of the component to the insulative material 50, the resultant charge at the gate or gate insulator 52 produces a voltage sensitivity when referenced to the substrate that is on the order of the 30 Nernstian Response (60 mV/decade/equivalent charge).

Turning now to a more detailed description of the conditioning circuit 36 illustrated in FIGURE 4, the function of conditioning circuit 36 is to condition the electronic response of IGFET 40 to the action of binding protein 32, so that the response is more easily received and analyzed by

-10-

processing circuit 26. Because of the relatively small variations in the charge or other electronic signal produced at the gate insulator 50 by the operation of binding protein 32, conditioning circuit 36 must be responsive to relatively slight input variations. Further, because information in digital form is frequently more 5 easily stored and processed by subsequent circuitry, conditioning circuit 36 preferably converts the response of IGFET(s) 40 into digital form.

In light of the foregoing observations, the use of unstably operated flip-flops in conditioning circuit 36, as briefly described below, is preferred. Additional detail concerning flip-flops operated in this manner is 10 provided by D. Lian and S. Middelhoek in Flip-Flop Sensors: A New Class Of Silicon Sensors, 9 Sensors and Actuators, 259-268 (1986). As shown in FIGURE 6, at least one flip-flop 56 is employed. Flip-flop 56 may be of the reset-set type commonly designated RS and constructed, for example, with two cross-coupled NOR gates having inputs designated R and S and producing outputs designated Q and Q. 15

Basically, the flip-flop 56 has two stable states. In one stable state, the Q output is high and the Q output is low. In the other stable state, the Q output is high and the Q output low. If an abrupt voltage pulse is applied to the S input of flip-flop 56, flip-flop 56 is brought into an unstable state. With the 20 flip-flop 56 in this unstable state, it is highly sensitive to remaining or injected charges, asymmetries in the NOR gates, noise, and input signals.

Without the influence of input signals, the probability that the flip-flop 56 will transfer to one or the other of the stable states can be determined statistically. Then, with the IGFET 40 connected to the S input of 25 flip-flop 56, even an extremely low signal variation from IGFET 40 will have an easily measured effect on the probability of a particular stable state of flip-flop 56 occurring. More particularly, the number of ones produced at output Q, for example, can be counted over a period during which the flip-flop 56 is repeatedly brought into an unstable state at a high frequency. The ratio between 30 the number of high outputs at the Q output to the number of unstable states can then be used to indicate the response of IGFET 40 to the operation of binding element 32. As a result, accuracy is enhanced both by the sensitivity of each individual measurement produced in this manner and the ability to repeat that measurement a relatively large number of times within a short time frame.

The foregoing operation of IGFET 40 and flip-flop 56 can perhaps be more readily understood in connection with the diagram provided in FIGURE 6. As shown, IGFET 40 is connected with its drain 46 and source 44 in series with the S input of flip-flop 56. A voltage pulse from processing circuit 26 is then applied to the drain 46 of IGFET 40 and input R of flip-flop 56, which are electronically connected. As will be appreciated from the preceding discussion, the imbalance produced by the IGFET 40 as a result of the operation of binding element 32 effects the probability of either the Q or \bar{Q} output being high. These outputs are continuously monitored by processing circuit 26, which then compares them with the number of voltage pulses applied to sensor element 16 to determine the concentration of the component in solution. As will be appreciated, the comparison between outputs and voltage pulses may vary directly or inversely in proportion to the concentration being determined depending upon the particular configuration of the IGFET 40 employed, the binding protein 32 selected, and the charge of the component of interest.

Given the time delays involved in the logic elements employed in flip-flop 56, it may be desirable to control flip-flop operation through a clock input designated C_C . Further, the details of flip-flop 56 can be varied and J-K flip-flops, D flip-flops and master-slave flip-flops, for example, can be employed in place of the RS flip-flop illustrated. Regardless of the construction of flip-flop 56, however, it is preferably a solid-state device integrated with the IGFET 40 onto a single substrate. As is discussed in greater detail below, a plurality of such combined IGFETs 40 and flip-flops 56 can be used to provide a continuum of responses to a particular characteristic of a component in solution or to respond to various components, depending on the particular binding elements 32 selected.

The final section of sensor element 16 to be discussed is the binding reversal element 38. As suggested above, the particular binding element 32 selected can be induced to release the component bound to the insulative material 50 by increasing the temperature of the gate 52 to approximately 75°C. To produce the required temperature change, diffused or thin-film resistors 58 can be integrated with the other components or the sensor element 16. The number, location, and resistivity of resistors 58, as well as the voltage applied, determines their power loss and, hence, the heat transferred to sensor element 16.

-12-

While thermal binding reversal can also be achieved by the immersion of sensor element 16 in water of a sufficiently high temperature, or through the use of denaturing agents, the use of resistors 58 allows the release of components to be effected in situ under the automatic control of processing circuit 26. As will be discussed in greater detail below, this feature has numerous advantages when employed in automatic control circuitry. To enhance the efficiency of the reversal performed by resistors 58, or to allow a more complete reversal at lower temperature, the charge at the surface of gate 52 may also be reversed.

As will be appreciated, and as is discussed in greater detail below, sensor element 16 may include a plurality of IGFETs 40 and flip-flops 56. The insulative material 50 of each IGFET 40 may have the same or different binding elements 32 immobilized thereon and the binding coefficients K_D of the various immobilized binding elements 32 may be the same or different, as desired. For the purpose of illustration, the discussions of system 10 operation below will treat the sensor element 16 shown in FIGURE 1 as including a single IGFET 40, while the sensor element 16 depicted in FIGURES 2 and 7 will be considered as having five separate IGFET 40 and flip-flop 56 sections schematically designated A, B, C, D, and E.

Turning now to a more detailed discussion of the processing circuit 26 shown in FIGURE 3, reference is again had to FIGURE 4. As shown, information received from sensor element 16 is conditioned by an input interface 60 for transmission to a microprocessor 62. As noted previously, with the use of flip-flop 56, this information includes high and low flip-flop outputs produced in response to the voltage pulses applied by microprocessor 62 to the drain 46 of IGFET 40 and the S input of flip-flop 56 in accordance with program instructions stored in read-only memory (ROM) 64. A random-access memory (RAM) 66 stores the number of voltage pulses applied by microprocessor 62 to the sensor element 16 during a predetermined time interval, allowing microprocessor 62 to compute the percentage of times that a particular stable state of flip-flop 56 is achieved during that interval.

In accordance with further instructions stored in ROM 64, microprocessor 62 compares this percentage with data representing an empirically derived calibration curve, also stored in ROM 64. The calibration

-13-

curve is basically a plot of independently determined information correlating, for example, various percentages of high flip-flop 56 output states and the corresponding component concentrations. Thus, by referring to the data in ROM 64, microprocessor 62 is able to determine the concentration of the 5 component of interest in solution.

An output interface 68 provides the information concerning concentration to the display elements 30. In the embodiment shown in FIGURE 1, the physical display 24 includes an alphanumeric display 70, such as a light-emitting diode (LED) array or liquid crystal display (LCD), that provides a 10 representation of the concentration. A second alphanumeric display 72 indicates whether some threshold change in concentration has been monitored over a predetermined interval.

Information concerning the concentration computed by microprocessor 62 can also be applied to certain control elements 32 included as 15 part of a larger control system. As will be appreciated, when supplied with information about the concentration of a component in solution and whether that concentration has varied by a predetermined amount over a given interval, the control elements 32 are able to initiate changes in the processing of the solution to achieve some predetermined component concentration. Thus, in addition to 20 providing raw information to control elements 32 microprocessor 62 may instruct control elements 32 concerning the proper response to that information.

Via output interface 68, microprocessor 62 also provides energy to the binding reversal element 38 at intervals and levels determined in accordance with instructions from ROM 64. In this manner, the binding 25 element 32 is caused to release the component back into solution as described previously in connection with the discussion of sensor element 16.

As will be appreciated, the instructions stored in ROM 64 that govern microprocessor 62 operation can be entered or altered by the operator's use of input elements 28. These elements 28 include a keypad 72 such as 30 pressure-sensitive switches 74 that allow, for example, binding reversal to be initiated manually or automatically at predetermined intervals. Further, keyboard 72 allows the instrument console to be turned on and information entered into RAM 66 for use by microprocessor 62 in controlling the operation of measuring system 10.

-14-

Summarizing the operation of the measuring systems 10 described above, with respect to the system 10 shown in FIGURE 1, the sensor element 16 is exposed to a solution of interest at some location remote from console 12. The binding protein 32, which is receptive to a particular component of the solution and has a predetermined binding coefficient K_D , is provided on the gate insulator region 52 of IGFET 40. As the binding protein 32 couples the component in solution to the insulative material 50 of IGFET 40, the drain current established in IGFET 40 by processing circuit 26, via cable 18, varies as a function of, for example, the concentration of the component in solution. With IGFET 40 connected in series with one of the inputs of the flip-flop 56, an imbalance in the unstable operation of flip-flop 56 results. The microprocessor 62 monitors this imbalance via signals received through cable 18 and determines the concentration of the component in solution by referring to empirically derived calibration curves stored in ROM 64. Microprocessor 62 provides the appropriate output information to the user via the display elements 32 and offers control over a solution processing system via control elements 32. The sensor element 16 can then be effectively "cleared" by activating the binding reversal element 38, causing binding element 32 to release the component of interest.

In the arrangement shown in FIGURE 2, the sensor element 16 includes five separate IGFET 40 and flip-flop 56 sections designated A, B, C, D, and E. While the sensor element 16 may be exposed to the solution of interest at some location remote from console 12 and subsequently analyzed upon insertion into console 12, in a preferred arrangement the sensor element 16 is both exposed to the solution and analyzed by processing circuit 26 after insertion. In this regard, the mechanical engagement between console 12 and sensor element 16 provides a fluid tight seal around sensor element 16. A first port 65 provided on the console 12 allows the solution of interest to be introduced into console 12, exposing the binding elements 32 on sensor element 16. An exit port 67 then exhausts the solution from console 12.

As will be appreciated, regardless of the manner in which the sensor element 16 of FIGURE 2 is exposed to the solution of interest, sensor element 16 is electronically connected to the processing circuit 26 upon insertion into console 12. Processing circuit 26 controls and responds to information from

-15-

each section A, B, C, D, and E in substantially the same manner described above for the single IGFET 40 and flip-flop 56 arrangement. Keypad 72 and display 24 allow operator inputs and information outputs, respectively, to be effected.

As will be appreciated, numerous variations of the multiple
5 IGFET 40 and flip-flop 56 sensor element 16 can be employed to adapt measuring system 10 for use in particular applications. If the same binding element 32 having the same binding coefficient or affinity K_D is provided on the gate insulator region 52 of each IGFET 40, information from each IGFET 40 and flip-flop 56 can be used to simultaneously determine the concentration of the
10 component in solution and produce an average value for display. Alternatively, this configuration allows information to be extracted from certain IGFETs 40, while the operation of the binding elements 32 on other IGFET 40 gates 52 is reversed. For example, with the sensor element 16 shown in FIGURES 2 and 7 employed, the operation of the binding elements 32 on the IGFETs 40 in section
15 A can be reversed while information is extracted from sections B, C, D, and E. Then the operation of sections B, C, D, and E can be reversed sequentially while information is extracted from the other nonreversed sections. Although this technique requires some thermal isolation between adjacent IGFETs 40, it provides the possibility of maintaining continuous, updated, measurements of the
20 component concentration in solution, with some IGFETs 40 always collecting information. As will be appreciated, this energization and reversal process is controlled by microprocessor 62 in response to instructions stored in ROM 64.

In another embodiment of sensor element 16 employing multiple IGFETs 40 and flip-flops 56, different binding elements 32 may be attached to
25 the gates or gate insulators 52 of different IGFETs 40. If these binding elements 32 are selected to respond to different components in the solution, it is then possible for microprocessor 62 to analyze characteristics of different components of the solution simultaneously. For example, with the sensor element 16 shown in FIGURES 2 and 7 constructed to operate in this manner, the binding elements 32 immobilized on the insulating material 50 of the IGFETs 40 in each section A, B, C, D, and E allow section A to respond to a first component, section B to respond to a second component, section C to respond to a third component and so on. By employing a plurality of IGFETs 40 with each

-16-

binding protein 32 selected, it is further possible to provide the continuous analysis described above for each component.

In another embodiment of the multiple IGFET 40 and flip-flop 56 sensor element 16, binding elements 32 for the same components are immobilized upon the insulative material 50 of each IGFET 40 but the binding coefficient K_D is different for each of the binding elements 32. As will be appreciated, when a sensor element 16 constructed in this manner is exposed to a solution containing the component of interest, the degree to which the component is bound to the gate region 52 of a particular IGFET 40 will be a function of the binding coefficient K_D associated with the corresponding binding element 32. Thus, a series of incremental responses will be received by microprocessor 62 from the various IGFETs 40 and flip-flops 56.

Addressing this arrangement in greater detail, with the sensor element 16 shown in FIGURES 2 and 7 constructed in this manner, the five sensor sections A, B, C, D, and E each include at least one IGFET 40. If the binding coefficient K_D of the binding proteins 32 immobilized on the insulative material 50 of the IGFETs 40 decreases with each consecutive section A, B, C, D, and E, the magnitude of the response of the IGFETs 40 in each section to changing component concentration will be as shown in FIGURE 8. While the curves shown are sigmoidal, the usual response vs. concentration curves are hyperbolic in nature. As indicated, the magnitude of the response at each section includes a relatively linear region during which an increase in concentration causes a corresponding increase in the magnitude of the section's response. Beyond this linear region, however, a saturated region exists in which increasing concentration of the component in solution does not substantially affect the magnitude of the section's response.

As will be appreciated, the response of an IGFET 40 in any particular section is determined by monitoring the drain current I_D . With flip-flops 56 employed, this becomes a matter of comparing the number of high outputs received with the number of voltage pulses applied to IGFET 40. Alternatively, the analog value of the drain current I_D can be measured directly by processing circuit 26. While I_D is actually inversely proportional to concentration in the linear region of each section's response, the following

-17-

discussion, as well as FIGURE 8, treats the magnitude of the various sections' response positively for convenience.

Whether the response of sensor section A, B, C, D, and E is produced via digital or analog hardware, the resultant information can be analyzed in several fashions. First, a "digital" mode of analysis will be addressed. In this mode of operation, microprocessor 62 receives appropriately conditioned information concerning the response of each sensor section A, B, C, D, and E. With reference to FIGURE 8, if sensor element 16 is exposed to a solution having a component concentration designated X, the response of section 5 E will fall within the saturated region and the section D response will be within the linear region. The remaining sections A, B, and C will be relatively unaffected. By requiring that the response of a section reach some predetermined threshold close to saturation before an output is produced, microprocessor 62 will only produce a high output for section E.

15 Now, if the concentration of the component in solution is increased to Y, the operation of sections B, C, D, and E will all be within the saturated region and only section A will be operating in the linear region. As a result, microprocessor 62 will produce high outputs corresponding to sections B, C, D, and E of sensor element 16.

20 In this manner an effectively digital analysis of the response of sensor element 16 is achieved. More particularly, if a high output corresponding only to the operation of section E is produced, a relatively low concentration is indicated. If high outputs for sections D and C are also produced, an intermediate concentration is indicated. When sensor element 16 is exposed to a 25 solution including the component of interest in relatively high concentration, high outputs for all five sections will result.

Thus, by appropriately selecting the binding coefficient K_D of the various binding elements 32, as well as the number of sections and relative variation between the various binding elements 32, substantially any quantization 30 of the sensor element response can be achieved. Further, with a sufficiently large number of IGFETs 40 employed, certain error desensitization techniques can be employed. More particularly, if sensor element 16 included thirty sections and the response of sections ten through thirty was high, with the exception of section twenty-seven, microprocessor 62 could be instructed to

-18-

reject the output of section twenty-seven as being most likely erroneous. As will be appreciated, with a single section employed such a correction would not be possible.

Because the accuracy of this digital operation is a function, in part, of the number of sections employed, if the sensor element 16 only includes a few sections it may be desirable to enhance the accuracy of the resultant measurement by switching microprocessor 62 to an "analog" mode of analysis. In this mode of operation, if the concentration of the component in solution is, for example, X, as shown in FIGURE 8, microprocessor 62 can use the information from section D to determine the corresponding concentration by referring to empirically derived calibration curves stored in ROM 64. Thus, even though the response of section D might be inadequate to initiate a digital output from microprocessor 62, in the analog mode of operation, valuable information is obtained enhancing accuracy.

Regardless of the operation of sensor element 16, a system 10 constructed in accordance with this invention does not require the use of external standard calibration references. More particularly, given the precise nature of the K_D of the binding elements 32, the relationship between the output of sensor element 16 and the concentration of the component in solution does not require adjustment between sensors.

As will be appreciated, a measuring system 10 constructed in the preceding manner has numerous applications. In the medical industry, system 10 can be used for blood and urine analysis. One specific example of a digital detector employs monoclonal antibodies directed against a drug. The binding constants of the monoclonal antibodies are determined by equilibrium dialysis. Antibodies with the appropriate affinities are used to construct a detector that covers the entire range of drug levels found in physiological fluids. In various industries, including the food industry, system 10 can be used for process monitoring and system/quality control. In agriculture, soil and fertilizer analysis can be performed by system 10. The monitoring and management of waste streams provides an environmental application for system 10.

Those skilled in the art will recognize that the embodiments disclosed herein are exemplary in nature and that various changes can be made therein without departing from the scope and spirit of the invention. In this

regard, and as was previously mentioned, the invention is readily embodied with various semiconductor configurations. Further, the information produced can be analyzed in either analog or digital form. Thus, an analog-to-digital converter could be employed in place of the flip-flops described or the analog output of the IGFET could be analyzed directly. Numerous configurations of the sensor can also be provided to enhance the utility of the measuring instrument with respect to a particular application. Because of the above and numerous other variations and modifications that will occur to those skilled in the art, the following claims should not be limited to the embodiments illustrated and discussed herein.

-20-

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A system for detecting a characteristic of a component of a medium comprising:

a sensor including detection means for responding electronically when exposed to said component and binding means for binding said component to said detection means; and

5 processing means, coupled to said sensor, for producing an output indicative of said characteristic of said component of said medium.

2. The system of Claim 1, wherein said binding means reversibly binds said component to said detection means.

3. The system of Claim 2, wherein said characteristic of said component of said medium detected by said instrument is its concentration.

4. The system of Claim 2, wherein said medium is a solution.

5. The system of Claim 4, wherein said binding means of said sensor comprises an organic ligand binder.

6. The system of Claim 5, wherein said detection means of said sensor comprises a semiconducting material.

7. The system of Claim 6, wherein said semiconducting material comprises at least one field-effect transistor having a gate insulator region, source region, and drain region, said organic ligand binder being coupled to said gate insulator region, said processing means applying an electronic signal between said source region and said drain region, the binding of said component to said gate insulator region influencing said signal, said processing means responding to the influence of said component on said signal to produce said output indicative of said characteristic of said component.

-21-

8. The system of Claim 7, wherein said semiconducting material comprises a plurality of said field-effect transistors, a separate said organic ligand binder being coupled to said gate insulator region of each said field-effect transistor.

9. The system of Claim 8, wherein each said separate organic ligand binder has a different binding coefficient with respect to said component of said solution.

10. The system of Claim 9, wherein said processing means applies an electronic signal between said source region and said drain region of each said field-effect transistor, the binding of said component to said gate insulator region of each said field-effect transistor influencing each said electronic signal in a manner functionally related to the binding coefficient of said organic ligand binder coupled to the corresponding said gate insulator region, said processing means responding to the influence of said component on each said signal to produce said output indicative of said characteristic of said component.

11. The system of Claim 8, wherein each said separate organic ligand binder reversibly binds a different said component to said semiconducting material.

12. The system of Claim 11, wherein said processing means applies electronic signals between said source region and said drain region of each said field-effect transistor, the binding of said components to said insulator regions of said field-effect transistors influencing each said electronic signal, said processing means responding to the influence of said components on each said signal to produce said output indicative of the characteristic of each said component.

13. The system of Claim 6, wherein said semiconducting material further comprises a resistor for producing a temperature increase in

-22-

said semiconducting material that causes said organic ligand binder to free a component previously bound to said semiconducting material.

14. The system of Claim 6, wherein said semiconductor material further comprises means for effecting a charge reversal in said semiconducting material that causes said organic ligand binder to free a component previously bound to said semiconducting material.

15. The system of Claim 6, wherein said processing means further comprises a microprocessor for monitoring said signal and producing said output indicative of the characteristic of said component by referring to empirically derived data correlating the characteristic with the signal level, and
5 display means for producing a visual representation of the characteristic of said component.

16. The system of Claim 15, wherein said microprocessor further produces a control signal indicative of the change in said solution required to achieve a predetermined characteristic of said component.

17. A method of detecting a characteristic of interest of a component of a medium comprising the steps of:

binding said component to a semiconducting element that responds electronically to said component;

5 monitoring the electronic response of said semiconducting element to said component; and

producing an output indicative of said characteristic of interest, said output being a function of the electronic response monitored.

18. The method of Claim 17, wherein said step of binding said component to said semiconducting element comprises the step of reversibly binding said component to said semiconducting element.

19. The method of Claim 18, wherein said step of reversibly binding said component to a semiconducting element further comprises the steps of:

immobilizing an organic ligand binder on a semiconducting
5 material; and

exposing said immobilized organic ligand binder to said medium,
said organic ligand binder reversibly binding said component to said
semiconducting element.

20. The method of Claim 19, wherein said medium is a
solution.

21. The method of Claim 20, wherein said characteristic of
interest is the concentration of said component in said solution.

22. The method of Claim 20, wherein said semiconducting
element comprises a field-effect transistor having gate insulator, drain and
source regions, said organic ligand binder being immobilized on said gate
insulator region.

23. The method of Claim 22, wherein said step of monitoring
the electronic response of said field-effect transistor comprises the step of
applying an electric potential between said drain and source regions to induce a
current therebetween and the step of determining the affect said component
5 bound by said organic ligand binder to said gate insulator region has upon said
current.

24. The method of Claim 21, further comprising the step of
reversing the binding of said component by said organic ligand binder.

25. The method of Claim 24, wherein said step of reversing
comprises the step of heating said semiconducting element.

26. The method of Claim 25, wherein said step of heating
said semiconducting element comprises the step of applying a voltage to a
resistor included in said semiconducting element.

-24-

27. The method of Claim 25, wherein said step of heating said semiconducting element comprises the step of exposing said organic ligand binder to warm water.

28. The method of Claim 24, wherein said step of reversing comprises the step of exposing said organic ligand binder to a denaturing agent.

29. The method of Claim 21, further comprising the step of producing a control signal in response to said characteristic of said component in said solution, said control signal being usable to effect a change in said solution.

30. A system for detecting a characteristic of at least one component of a medium, said system comprising:

a sensor including detection means for responding electronically when exposed to said component and a plurality of binding means for binding said component to said detection means; and

processing means, coupled to said sensor, for producing an output indicative of said characteristic of said component of said medium.

31. The system of Claim 30, wherein said binding means reversibly bind said component to said detection means.

32. The system of Claim 31, wherein said detection means includes a plurality of detection elements, upon which said binding means are immobilized, each said detection element responding electronically to the binding of said component to said detection element by said binding means immobilized thereon.

33. The system of Claim 32, wherein said binding means immobilized on separate said detection elements bind different components of said medium to said detection elements, said processing means producing outputs indicative of the characteristics of each said component of said medium.

-25-

34. The system of Claim 32, wherein said binding means immobilized on separate said detection elements bind the same components of said medium to said detection elements, but have different binding coefficients.

35. The system of Claim 34, wherein said processing means produces a digital output corresponding to the electronic response of each said detection element, prior to producing said output indicative of said characteristic of said component.

36. The system of Claim 35, wherein said processing means rejects said digital outputs that are determined to be in error.

37. A method of detecting a characteristic of at least one component of a medium, said method comprising the steps of:

binding said component to a plurality of detection elements that respond electronically to said component;

5 monitoring the electronic response of said detection elements to said component; and

producing an output indicative of said characteristic of interest, said output being a function of the electronic response monitored.

38. The method of Claim 37, wherein said step of binding said component to said detection elements comprises the step of reversibly binding said component to said detection elements.

39. The method of Claim 38, wherein the step of binding said component to said detection elements comprises the step of binding different said components to different said detection elements, said step of producing an output indicative of said characteristic of said component comprising producing 5 separate outputs indicative of each said component bound to a different detection element.

40. The method of Claim 38, wherein the step of binding said component to said detection elements comprises the step of binding said component to different said detection elements in different degrees.

-26-

41. The method of Claim 40, wherein said step of producing an output indicative of said characteristic of said component further comprises the step of producing a digital output for each said detection element as a function of the electronic response of said detection element to the binding of
5 said component.

42. The method of Claim 41, further comprising the step of rejecting said digital outputs determined to be in error.

43. A system for detecting a characteristic of at least one component of a medium, said system comprising:

a sensor including a plurality of detector elements for responding electronically when exposed to said component and a plurality of binding means
5 for binding said component to said detection elements, each said binding means being selectively responsive to a specific range of said component characteristic and being immobilized on separate said detection elements, each said detection element responding electronically to the binding of said component to said detection element; and

10 processing means coupled to said sensor, for producing a digital output corresponding to the electronic response of each said detection element and analyzing said digital outputs to determine said characteristic of said component without reference to an external calibration standard.

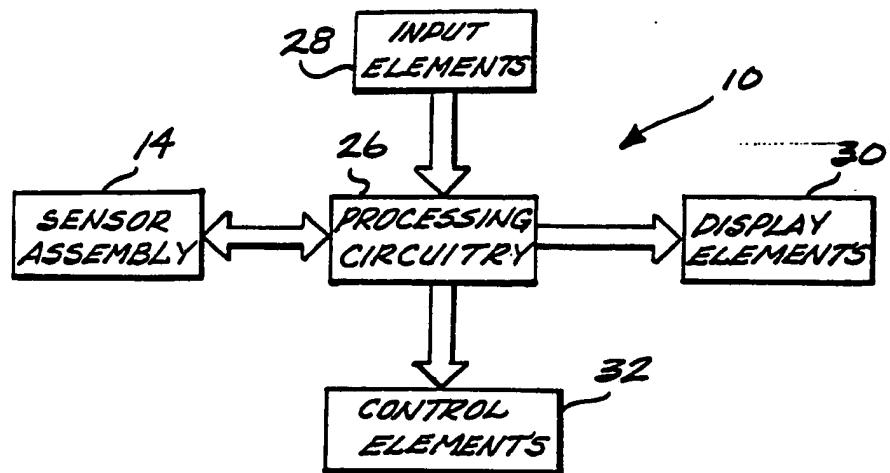
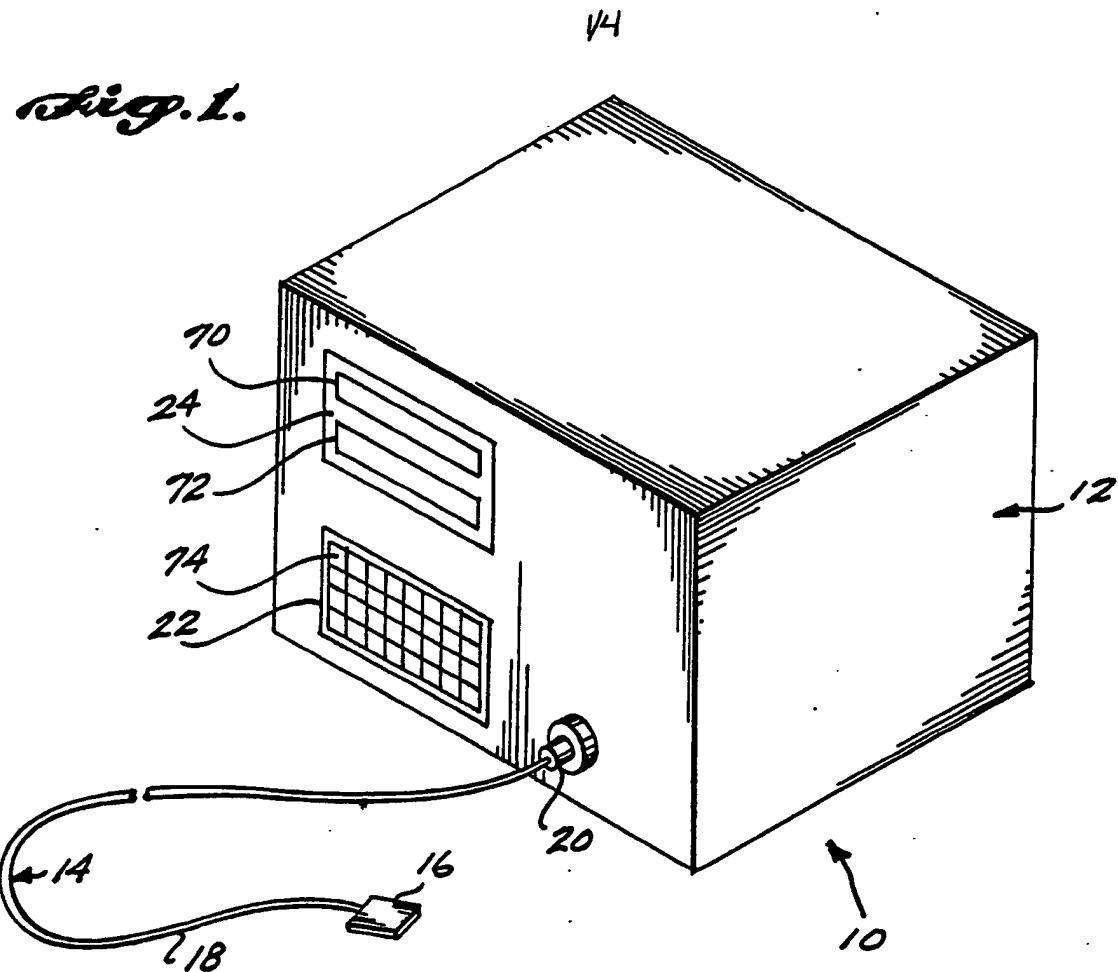


fig. 3.

2/4

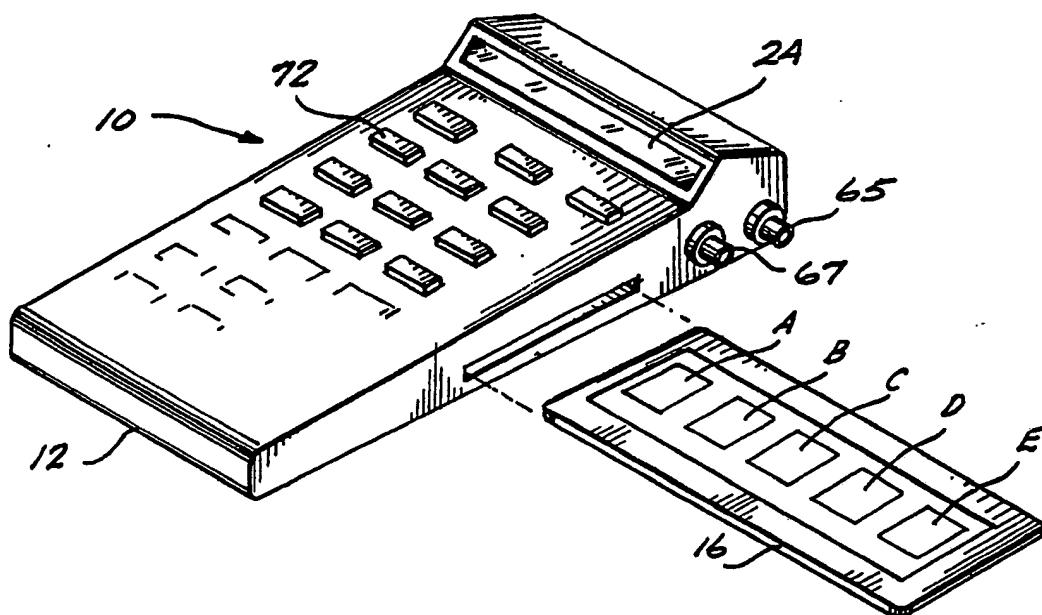


fig. 2.

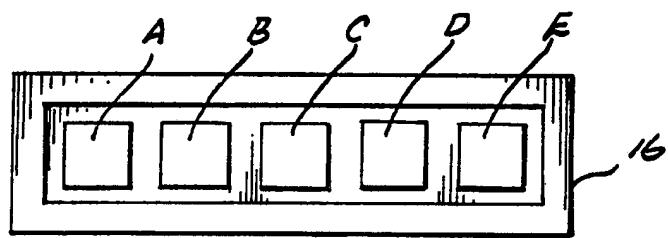


fig. 7.

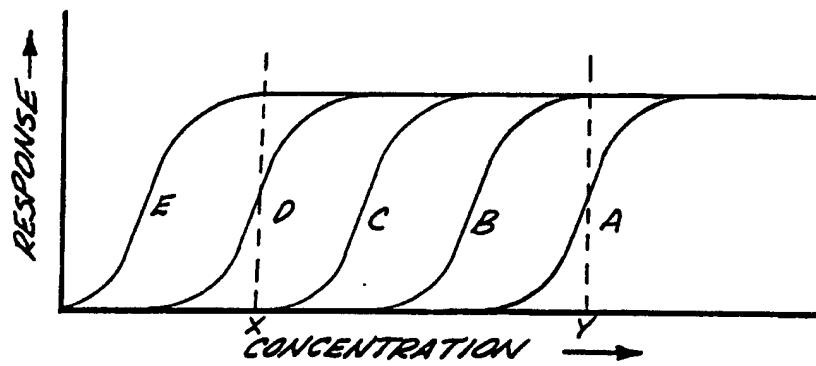
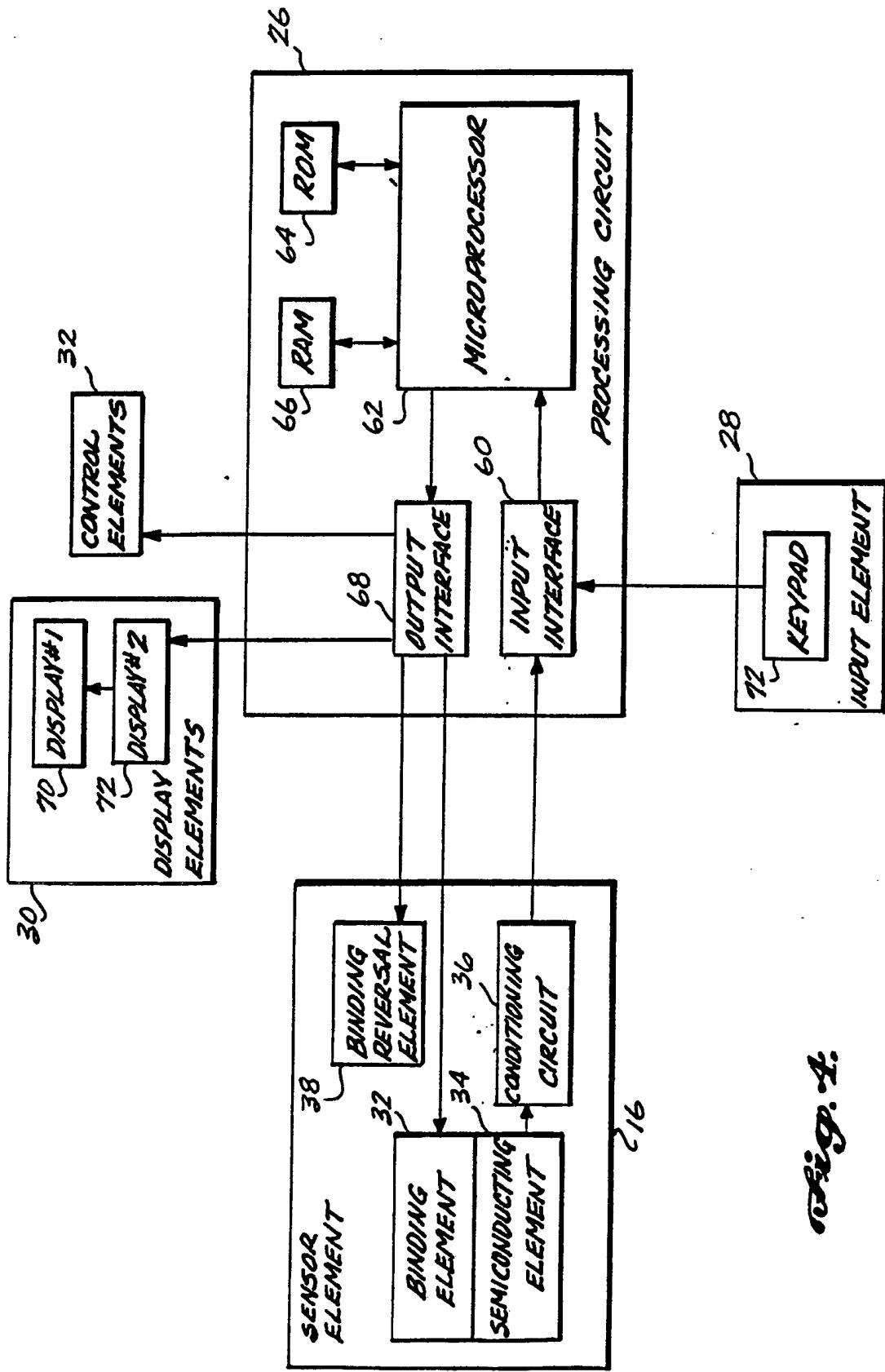


fig. 8.

3/4



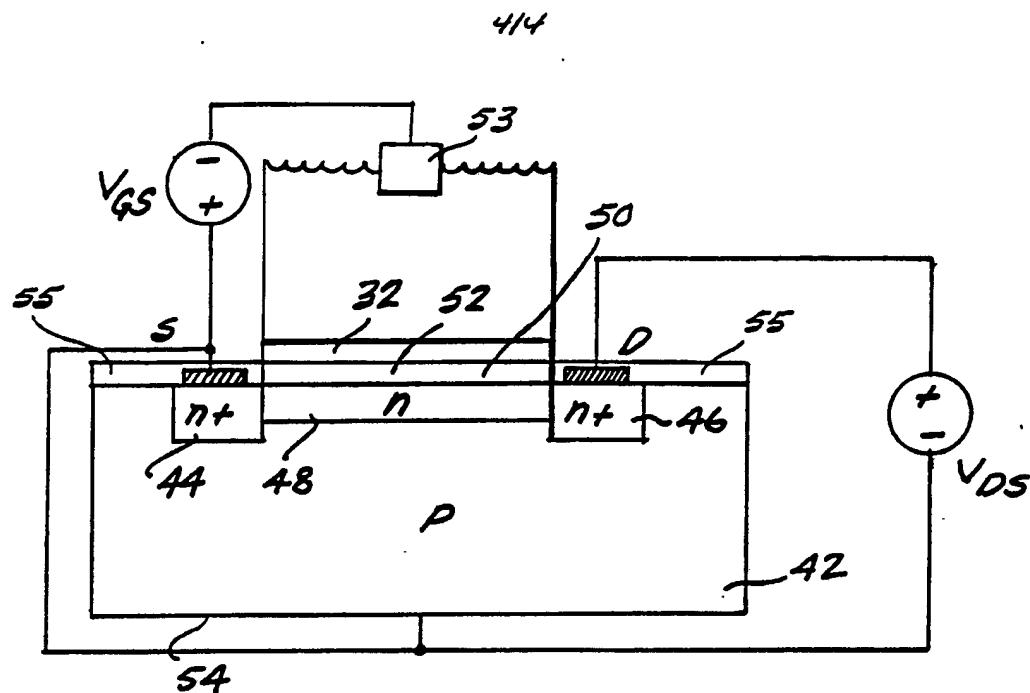
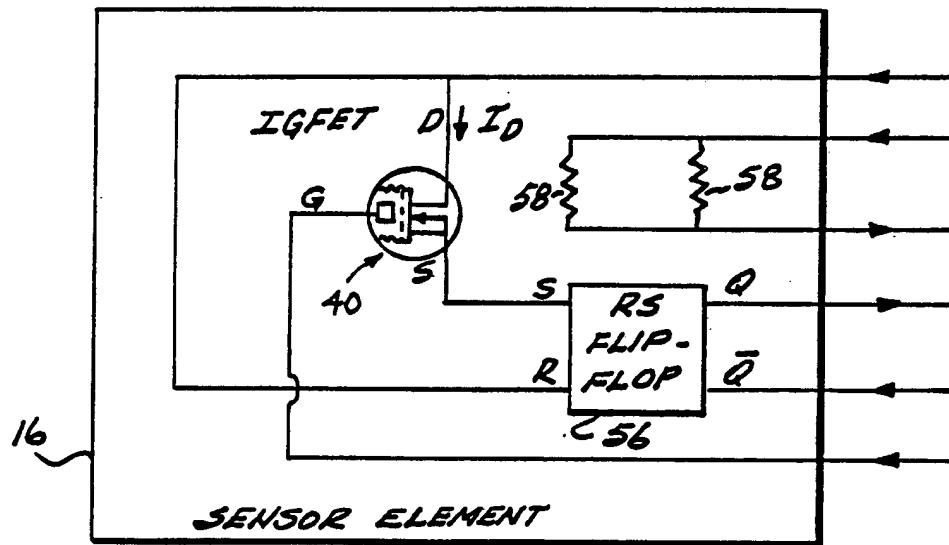


FIG. 5.

FIG. 6.
SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 88/01494

I. CLASSIFICATION & SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC⁴ : G 01 N 27/00

II. FIELDS SEARCHED

Minimum Documentation Searched ⁷

Classification System	Classification Symbols
IPC ⁴	G 01 N

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁸

III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹

Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
Y	EP, A, 0214805 (K. OGAWA) 18 March 1987 see page 4, line 11 - page 6, line 14	1-12,15, 17-25,30- 35,37,41, 43
Y	-- US, A, 4238757 (J.F. SCHENCK) 9 December 1980 see column 3, line 31 - column 4, line 21; figure 1	1-12,15, 17-25,30- 35,37,41, 43
Y	-- US, A, 3831432 (P.F COX) 27 August 1974 see column 3, line 59 - column 4, line 39; column 7, lines 6-40	1-12,15, 17-25,30- 35,41,43
A	-- FR, A, 2304083 (UNIVERSITY OF UTAH) 8 October 1976 see figure 2	1
A	-- US, A, 4334880 (M.K. MALMROS) 15 June 1982 see front-page	1
	--	. / .

- * Special categories of cited documents: ¹⁴
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "A" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

13th September 1988

Date of Mailing of this International Search Report

= 7 OCT 1988

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer

P.C.G. VAN DER PUTTEN

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FR M THE SEC ND SHEET)		
Category*	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	Patent Abstracts of Japan, volume 8, no. 254 (P-315)(1691), 21. November 1984 & JP, A, 59125054 (KURARAY K.K.) 19 July 1984 see abstract --	1
A	WO, A, 85/04480 (R. HOIJTINK) 10 October 1985 see front-page --	1
A	Sensors and Actuators, volume 9, 1986, Elsevier Sequoia, (NL), W.J. Lian et al.: "Flip-flop sensors: a new class of silicon sensors", pages 259-268 cited in the application -----	

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. US 8801494
SA 22264

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
 The members are as contained in the European Patent Office EDP file on 29/09/88
 The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A- 0214805	18-03-87	JP-A-	62050656	05-03-87
		JP-A-	62050657	05-03-87
US-A- 4238757	09-12-80	None		
US-A- 3831432	27-08-74	None		
FR-A- 2304083	08-10-76	NL-A- BE-A- DE-A,C US-A,B AU-A- CH-A- GB-A- JP-A- AU-B- CA-A- SE-A- SE-B-	7602619 839557 2610530 4020830 1193576 600334 1529743 51139289 501668 1059645 7603229 428607	14-09-76 13-09-76 23-09-76 03-05-77 15-09-77 15-06-78 25-10-78 01-12-76 28-06-79 31-07-79 13-09-76 11-07-83
US-A- 4334880	15-06-82	US-A-	4444892	24-04-84
WO-A- 8504480	10-10-85	NL-A- EP-A- JP-T- US-A-	8400916 0177544 61501726 4735702	16-10-85 16-04-86 14-08-86 05-04-88